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Linkage and association analysis of obesity traits reveals novel loci and interactions with dietary n-3 fatty acids in an Alaska Native (Yup'ik) population

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ABSTRACT

Objective. To identify novel genetic markers of obesity-related traits and to identify gene-diet interactions with n-3 polyunsaturated fatty acid (n-3 PUFA) intake in Yup'ik people.

Material and methods. We measured body composition, plasma adipokines and ghrelin in 982 participants enrolled in the Center for Alaska Native Health Research (CANHR) Study. We conducted a genome-wide SNP linkage scan and targeted association analysis, fitting additional models to investigate putative gene-diet interactions. Finally, we performed bioinformatic analysis to uncover likely candidate genes within the identified linkage peaks.

Results. We observed evidence of linkage for all obesity-related traits, replicating previous results and identifying novel regions of interest for adiponectin (10q26.13-2) and thigh circumference (8q21.11-13). Bioinformatic analysis revealed *DOCK1*, *PTPRE* (10q26.13-2) and *FABP4* (8q21.11-13) as putative candidate genes in the newly identified regions. Targeted SNP analysis under the linkage peaks identified associations between three SNPs and obesity-related traits: rs1007750 on chromosome 8 and thigh circumference (P = 0.0005), rs878953 on chromosome 5 and thigh skinfold (P = 0.0004), and rs1596854 on chromosome 11 for waist circumference (P = 0.0003). Finally, we showed that n-3 PUFA modified the association

Abbreviations: BMI, body mass index; GWAS, genome-wide association study; n-3 PUFA, omega-3 polyunsaturated fatty acids; CANHR, Center for Alaska Native Health Research; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; $\delta^{15}N$, nitrogen stable isotope ratio; RBC, red blood cell; SNP, single nucleotide polymorphism; LOD, logarithm of the odds.

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between obesity related traits and two additional variants (rs2048417 on chromosome 3 for adiponectin, P for interaction = 0.0006 and rs730414 on chromosome 11 for percentage body fat, P for interaction = 0.0004).

Conclusions. This study presents evidence of novel genomic regions and gene-diet interactions that may contribute to the pathophysiology of obesity-related traits among Yup'ik people.

1. Introduction

Obesity is a complex disorder arising from multiple interactions of genes, behavior and environment. Family-based heritability estimates provide strong evidence of genetic contributions to obesity-related phenotypes [1–3]. Although more than 40 genome-wide linkage scans and association studies, as well as several hundred candidate gene studies, have yielded numerous obesity-related loci, a large proportion of the genetic risk for obesity remains unexplained [3–5]. While a few genomic regions, e.g., 1p36, 2p21, 3q27, 10p12, and 11q23-24 or genes such as *FTO* and *MC4R* have been widely replicated across various studies, other findings remain largely inconsistent [3,4,6]. Moreover, body mass index (BMI) is an imperfect proxy for body fat with differential validity among populations, which highlights the importance of studying additional phenotypes such as skinfold thickness or circumference measures [2].

Meta-analyses of genome wide association studies (GWAS) and linkage scans suggest that obesity-related phenotypes may be influenced by many genes with small effects [7]. Such genes can be fruitfully studied in geographically remote populations, such as the Yup'ik people, due to their extended family structure and reduced genetic variation [8]. Yup'ik people living in Southwest Alaska have a high prevalence of the 'healthy obese' phenotype, where obesity is not closely tied with other metabolic complications as is commonly seen in other populations [9]. Specifically, Yup'ik people have a historically low prevalence of insulin resistance, metabolic syndrome and type 2 diabetes despite obesity prevalence similar to those of the US and Canadian general populations [10–15]. Such unique separation of obesity with other metabolic complications may be related to the traditional Yup'ik diet, which is high in marine-derived omega-3 polyunsaturated fatty acids (n-3 PUFA) [9,16]. Although prior studies from our group have found associations between candidate SNPs in *FTO*, *ETV5*, *HHEX*, *CDKAL1* and metabolic traits [17,18], the genetic contributions to obesity in the Yup'ik have not been comprehensively examined at the genome-wide level.

In this study, we examined the genetic architecture of obesity-related phenotypes, including BMI, multiple measures of body composition, and plasma adipokine concentrations, in Yup'ik people. We conducted a whole genome linkage scan and targeted association testing within observed linkage peaks, supplemented by a bioinformatic analysis. In addition, we investigated whether a diet rich in marine n-3 PUFA intake modifies the associations between genetic variants and obesity traits.

2. Material and Methods

2.1. Participants

The Center for Alaska Native Health Research (CANHR) studies genetic, behavioral, and dietary risk factors underlying obesity and their relationship to diabetes and cardiovascular disease among Yup'ik people [15]. Recruitment of participants from 11 Southwest Alaska communities began in 2003 and is currently ongoing. This study sample consists of 982 non-pregnant Yup'ik individuals that were ≥14 years old at the time of enrollment and reflects the age distribution of all eligible participants according to the 2000 U.S. census.

2.2. Ethics

Participants provided written informed consent. The Institutional Review Boards of the University of Alaska and the National and Alaska Area Indian Health Service, as well as the Yukon-Kuskokwim Human Studies Committee, approved the study protocols.

2.3. Measurements of Anthropometric, Clinical, and Dietary Characteristics

Anthropometric measurements, including height, weight, four circumferences (waist, hip, triceps, and thigh), and four skinfolds (abdominal, subscapular, triceps and thigh) were collected using protocols from the National Health and Nutrition Examination Survey III Anthropometric Procedures Manual [19] as previously described [14] and percentage body fat was measured by electrical bioimpedance using a Tanita TBF-300A body composition analyzer (Tanita, Arlington Heights, IL). Blood samples were collected from participants after an overnight fast, and lipoproteins including total cholesterol, high density lipoprotein (HDL-) cholesterol, low density lipoprotein (LDL-) cholesterol, very low density lipoprotein (VLDL-) cholesterol, apolipoprotein A1, and plasma triglycerides were assayed as previously described [14]. Adiponectin and leptin were measured by radioimmunoassay (Linco Research, St Charles, MO for adiponectin and leptin; and Phoenix Pharmaceuticals, Burlingame, CA for ghrelin). Intra- and inter-assay coefficients of variation were respectively 7.1% and 12.1% for adiponectin, 6.7% and 11.1% for leptin, and 3.4% and 25.4% for ghrelin [20]. Long-term intake of n-3 PUFA was estimated using the nitrogen stable isotope ratio ($\delta^{15}N$) of red blood cells (RBC), as previously described [21–23].

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2.4. Genotyping and Statistical Analysis

The Linkage-IV panel (Illumina, San Diego, CA) was used to genotype 6090 single nucleotide polymorphisms (SNPs) at the Center for Inherited Disease Research [22]. Family structure, quality control procedures, and methods used for both linkage and association analysis are described in detail previously [22]. Briefly, for non-normally distributed traits, we implemented either logarithmic transformation for body fat proportion, waist and thigh circumferences, abdominal, subscapular, and triceps skinfolds, leptin, and ghrelin; square root transformation for adiponectin; and Box–Cox transformation for hip and triceps circumferences to normalize the distributions of residuals [24]. All linkage and association models included age, sex, community group (inland vs. coastal), and n-3 PUFA intake. In the association analyses, we fit additional models including an interaction term between the SNP genotype (additive effect) and quartiles of the $\delta^{15}N$, and used likelihood ratio test to compare the models. We estimated statistical power to detect SNP-$\delta^{15}N$ interactions to exceed 60% for the examined traits. To correct for multiple testing, we determined the effective number of SNPs using spectral decomposition of the correlation matrix for the SNP genotypes [25,26]. We annotated SNPs using the SNAP 2.2 (SNP Annotation and Proxy Search) program, the 1000 Genomes Pilot project data, and GeneCruiser 3.2.2 [27,28]. To identify SNPs occurring in potential regulatory regions, we used RegulomeDB [29].

2.5. Bioinformatic Analysis

We searched for the keyword "obesity" to compile a list of training genes using the Gene Evidence search in HUGE Navigator [30], selecting only genes with a HUGE score of 0.10 that were only related to obesity and not type 2 diabetes. We converted gene names to Ensembl or EntrezGene IDs using BioMart Central Portal ID converter [31]. Information on genes of interest was obtained using GeneCards [32].

Using our study data, we extracted lists of genes that physically fell within linkage peaks that exceeded a logarithm of the odds (LOD) score of 2. To identify obesity candidate genes from these regions, we employed three complementary software packages, which use different prioritization methodologies: Gene Wanderer [33], TOPPGene Suite [34], and ENDEAVOUR [35]. Gene Wanderer prioritizes genes by using random walk analysis to estimate similarities in protein-protein interaction networks [33]. TOPPGene uses semantic annotations to estimate fuzzy-based similarity measures between any two genes that are subsequently combined into an overall score; a P-value of each annotation of a test gene is then derived by random sampling of the whole genome [34]. We chose the default gene model for TOPPGene analysis. ENDEAVOUR uses a set of training genes and multiple genomic data sources to first propose several models, then applies each model to the candidate genes to rank them against the profile of the training genes and estimate P-values, and finally merges the rankings from each model into one global ranking [35]. Our ENDEAVOUR model included BIND, MINT, BioGRID, STRING, BPRD and Intact interactions, KEGG and GO annotations, as well as Cis-regulatory models, motif and text mining. We based all coordinates on the human genome 18 build (Hg18), converting from build19 (Hg19) using UCSC Browser LiftOver tool when appropriate. We considered results from the analysis significant if the P-value (TOPPGene and ENDEAVOUR) was less than or equal to 0.005, or if the Gene Wanderer prioritization score, which reflects the global similarity of candidate genes to members of a known disease gene family [33], was at least 0.1. We defined candidate genes as genes that were identified by at least two out of the three algorithms, or by one algorithm plus interactions identified by Ingenuity IPA (Ingenuity Systems, Redwood City, CA).

3. Results

3.1. Baseline Characteristics

Table 1 presents demographic and clinical characteristics of our study sample. Females represented slightly more than half the sample, particularly in the inland communities (57%). The distribution of all obesity-related traits varied by gender (P-value < 0.05), with the exception of waist and triceps circumferences. All traits exhibited statistically significant heritability estimates, ranging from 28% for waist circumference and abdominal skinfold to 47% for adiponectin and triceps circumference (Table 2).

Table 1 – Demographic and anthropometric characteristics of the study sample.

	Male (n = 454)	Female (n = 528)	P-value
Community group, n (%) [a]			
Coastal	214 (51) [b]	204 (49)	0.63
Inland	240 (43)	324 (57)	0.0004
Age, years	36 (35-38)	38 (37-40)	0.072
$\delta^{15}N$, ‰	8.8 (8.7-8.9)	9.2 (9.1-9.3)	0.0001
BMI, kg/m^2	25.6 (25.2–26.0)	28.2 (27.7–28.8)	<0.0001
Percent body fat	21.2 (20.4–21.9)	35.5 (34.8–36.3)	<0.0001
Circumference, cm	44.7 (44.7–44.7)	44.8 (44.8–44.8)	<0.0001
Triceps			
Hip	30.4 (30.1–30.8)	30.4 (30.0–31.0)	0.822
Thigh	50 (49–50)	51 (50–51)	0.007
Waist	88 (87–90)	90 (89–91)	0.081
Skinfold, mm			
Abdominal	16 (15–17)	29 (28–30)	<0.0001
Subscapular	13 (12–13)	23 (22–24)	<0.0001
Triceps	11.2 (10.7–11.7)	25.6 (24.8–26.4)	<0.0001
Thigh	11.9 (11.3–12.5)	30.0 (29.1–31.0)	<0.0001
Adiponectin, mg/mL	9.0 (8.6–9.5)	9.9 (9.4–10.3)	0.009
Leptin, ng/mL	2.6 (2.4–2.8)	13.4 (12.6–14.3)	<0.0001
Ghrelin, pg/mL	37 (36–39)	40 (38–41)	0.01

[a] P-value was based on binomial distribution.

[b] For continuous variables, we report means of untransformed traits and 95% confidence intervals in parentheses; for the categorical variable of community group, we report counts and percentages in parentheses. P-values were based on a 2-sample t-test using transformed trait values.

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Table 2 – Heritability estimates of obesity-related traits.

	Heritability	P-value
Adiponectin	0.47	6.0×10^{-18}
Leptin	0.30	1.3×10^{-6}
Ghrelin	0.42	9.7×10^{-11}
BMI	0.41	1.2×10^{-8}
Circumference		
Triceps	0.47	2.6×10^{-11}
Hip	0.43	1.1×10^{-9}
Thigh	0.39	4.0×10^{-7}
Waist	0.28	4.5×10^{-5}
Percent body fat	0.31	5.5×10^{-6}
Skinfold		
Abdominal	0.28	4.3×10^{-6}
Subscapular	0.30	1.1×10^{-6}
Thigh	0.35	2.0×10^{-7}
Triceps	0.36	1.0×10^{-7}

3.2. *Genomewide Linkage Scan Results*

We present results for the genome-wide linkage scan in Table 3. We considered peaks that exceeded a LOD score of 2 or greater as significant. All of the traits, except leptin, showed evidence for areas of linkage (the highest LOD score for leptin was 1.9, just below our cutoff). Several traits shared linkage regions, including adiponectin and triceps skinfold (10q26.13-10q26.2); triceps skinfold, waist circumference, percent body fat, and BMI (11p15.1-11p13); waist circumference and percent body fat (4p16.1-4p15.1); and thigh and subscapular skinfold (5q32-5q42).

3.3. *Targeted Association Results*

Results of the targeted association testing of individual SNPs located in regions identified by genome wide linkage are presented in Table 4. Adiponectin, body fat, thigh circumference, thigh skinfold, and waist circumference each had a single SNP that showed significant associations. In models investigating plasma adiponectin and percent body fat, we observed statistically significant interactions between genotype and n-3 PUFA intake (Fig. 1).

3.4. *Bioinformatic Analysis Results*

Table 5 lists the top candidate genes for each of the linkage peaks identified in this study. At least one of the proposed candidate genes has been previously associated with obesity for almost all linkage peaks [36]. The exception is 10q26.13-10q26.2, which we found to be linked to adiponectin and triceps skinfold. In that region, TOPPGene was the only algorithm that identified potential candidate genes, *DOCK1* and *PTPRE*. Although neither *DOCK1* nor *PTPRE* has been previously associated with obesity traits, they are paralogs for known obesity susceptibility genes: *DOCK5* and *PTPRA*, *PTPRD*, and *PTPRF*, respectively [37,38].

4. Discussion

We conducted a genome-wide linkage scan and targeted SNP association analysis for obesity-related traits in a cross-

Table 3 – Observed maximum logarithm of the odds (LOD) score and linkage regions with LOD >2.

Chromosome bands	Peak LOD	Start SNP	Start position	End SNP	End position
Adiponectin					
3q26.33-3q29	3.65	rs2049769	181,136,696	rs711995	195,842,216
10q26.13-10q26.2	2.20	rs10899	125,758,210	rs1255135	130,007,627
22q13.1-22q13.31	2.12	rs137636	38,052,423	rs12170546	42,753,666
Triceps circumference					
11q24.1-11q25	2.54	rs676943	121,553,535	rs965493	131,145,305
BMI					
4p15.33-4p15.32	2.04	rs763318	12,572,672	rs1357233	16,752,474
11p15.4-11p15.3	2.64	rs892336	5,559,255	rs1365406	12,519,296
Percent body fat					
4p16.1-4p15.2	2.72	rs881641	9,742,845	rs1533132	24,921,455
11p15.4-11p15.1	2.79	rs906895	6,236,824	rs4348874	18,748,760
Ghrelin					
13q12.13-13q13.3	2.69	rs306395	25,348,564	rs737645	38,931,693
Subscapular skinfold					
5q33.1-5q34	2.23	rs1560657	149,971,205	rs7717940	161,694,190
Thigh circumference					
8q21.11-8q21.13	2.18	rs1007750	74,148,846	rs10105219	84,019,730
9q34.2-9q34.3	2.12	rs7023064	136,236,772	rs7357733	140,123,767
Thigh skinfold					
5q32-5q34	2.58	rs887346	149,576,185	rs1030154	164,983,908
Triceps skinfold					
10q26.13-10q26.2	2.14	rs4962480	127,378,272	rs1255135	130,007,627
11p15.1-11p13	2.21	rs1470251	20,078,168	rs509628	31,491,931
Waist circumference					
4p16.1-4p15.1	3.11	rs881641	9,742,845	rs902658	31,864,983
11p15.4-11p13	3.34	rs1451724	3,813,244	rs750780	33,874,170

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Table 4 – Associations between single nucleotide polymorphisms (SNPs) within linkage peaks and obesity related traits.

	SNP	Chr	Gene	P-value [a]	P-value [b]	P-value [c]
Adiponectin	rs2048417	3	–	0.58	0.001	0.0006
Percent body fat	rs730414	11	*BTBD10*	0.48	0.0009	0.0004
Thigh circumference	rs1007750	8	*SBSPON*	0.0005	0.0003	0.03
Thigh skinfold	rs878953	5	–	0.0004	0.003	0.30
Waist circumference	rs1596854	11	*LUZP2*	0.0003	0.002	0.30

[a] P-value for the model adjusted for sex, age, n-3 PUFA intake, community group, and additive genotype.
[b] P-value for the model with all the terms from the additive model plus an interaction between the additive genotype and n-3 PUFA intake.
[c] P-value for the likelihood ratio test for comparing the additive model with the full model (i.e. for the interaction between genotype and n-3 PUFA intake).

sectional sample of Yup'ik people. In agreement with previous studies of fat distribution genetics [39], we found distinct signals underlying different obesity phenotypes, e.g. different skinfold thicknesses. Several of our findings overlapped with known linkage peaks, previously identified candidate genes, or proposed candidate genes from mouse studies. Specifically, the 11p15.4-11p13 region, which was significantly linked with BMI, waist circumference, body fat percent and triceps skinfold, contains the obesity candidate gene *BDNF* (brain-derived neurotrophic factor, geneID 627). *BDNF* and its tyrosine kinase receptor are expressed in hypothalamic nuclei associated with eating behaviors [40] and have been linked to body weight and eating disorders in European populations [41]. Several *BDNF* variants were robustly associated with BMI in a large-scale GWAS comprised of Europeans and African Americans [42]. However, we have previously reported null associations between the nonsynonymous variant rs6265 in the *BDNF* gene and obesity phenotypes in Yup'ik people [17]. One reason for this discrepancy could be the low minor allele frequency (0.04) at

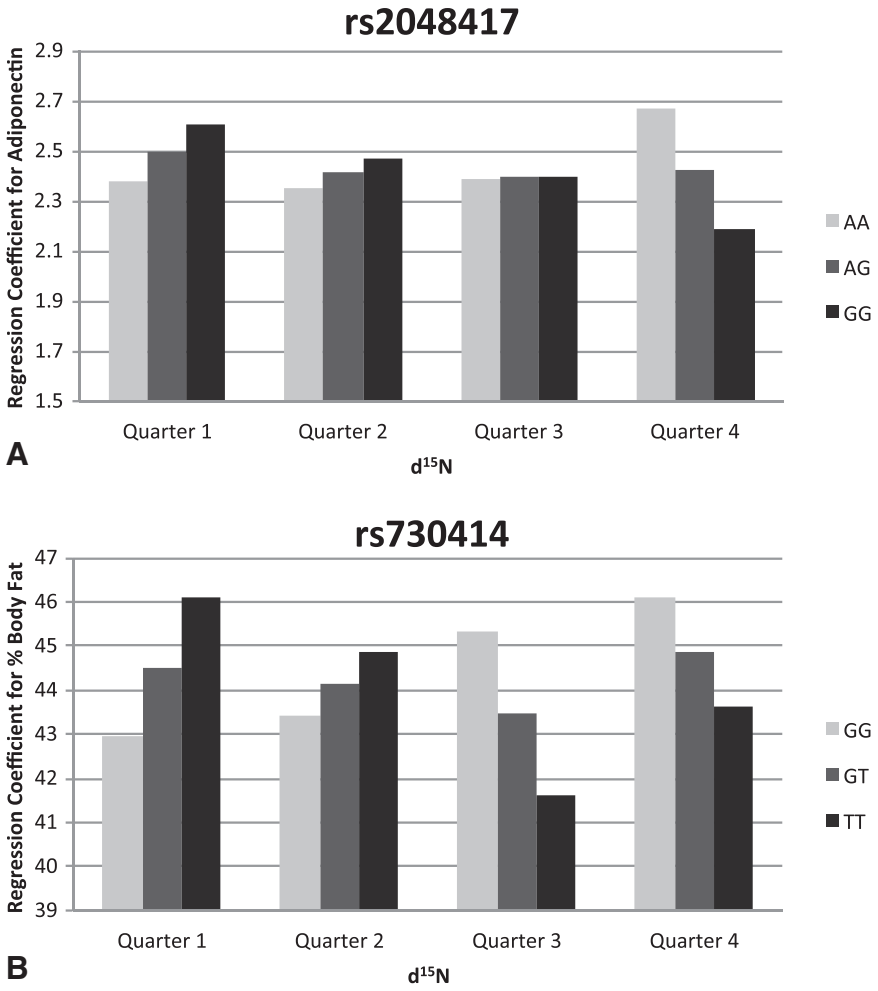

Fig. 1 – Interactions between genotype and the quarter of n-3 PUFA intake as measured by $\delta^{15}N$ for A) rs2048417 in the adiponectin analysis and B) rs730414 in the percent body fat analysis.

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Table 5 – Putative candidate genes from bioinformatic analysis.

Chromosome	Training list genes	Putative candidate genes
Adiponectin		
3q26.33-3q29	*ETV5*	
10q26.13-10q26.2		*DOCK1*, *PTPRE*
22q13.1-22q13.31		*EP300*, *SREBF2*
Triceps circumference		
11q24.1-11q25		*TIRAP*, *HSPA8*, *CBL*
BMI		
4p15.33-4p15.32		*PPARGC1A*
11p15.4-11p15.3	*BDNF*	
Percent body fat		
4p16.1-4p15.2		*PPARGC1A*
11p15.4-11p15.1	*BDNF*	
Ghrelin		
13q12.13-13q13.3		*PDX1*
Subscapular skinfold		
5q33.1-5q34		*ADRA1B*, *IL21B*
Thigh circumference		
8q21.11-8q21.13		*FABP4*
9q34.2-9q34.3		*RXRA*, *GRIN1*, *EHMT1*
Thigh skinfold		
5q32-5q34		*ADRA1B*, *IL21B*
Triceps skinfold		
10q26.13-10q26.2		*DOCK1*, *PTPRE*
11p15.1-11p13	*BDNF*	
Waist circumference		
4p16.1-4p15.1		*PPARGC1A*
11p15.4-11p13	*BDNF*	

the rs6265 locus in our study sample [17]. It is likely that the linkage peak observed in this study is driven by other variants in the 11p15.4-11p13 region. Other findings on chromosome 11 included a linkage peak for triceps circumference in the 11q24.1-11q25 region, located adjacent to but not overlapping with the previously reported peak for percent body fat in a Pima Indian population [43].

The linkage peak for adiponectin on chromosome 3 includes two known and biologically plausible candidate genes, namely *ETV5* (ets variant 5, geneID 2119) and *ADIPOQ* (adiponectin, C1Q and collagen domain containing, geneID 9307). While the role of *ADIPOQ* variation in metabolic phenotypes has long been established [44], evidence indicates that *ETV5* also merits attention. A polymorphism in *ETV5*, rs117016164, showed a robust association with circulating plasma adiponectin in Filipino women [45]. Another common variant in *ETV5*, rs7647305, was correlated with BMI and weight in a large-scale GWAS [42] as well as with hip and thigh circumference in Yup'ik people [17]. We also found an adiponectin peak on chromosome 10, located adjacent to a recently identified novel locus near *WDR11-FGFR2* that was linked to circulating adiponectin, blood lipids, and BMI-adjusted waist-to-hip ratio in East Asian populations [46]. Notably, the same 10q26.2 region showed robust evidence of linkage to plasma triglycerides in another Native American population: the Arizona subset of the Strong Heart Family Study [47]. Overall, our findings corroborate previously reported observations that the majority of adiponectin-linked loci have pleiotropic effects, particularly on circulating adiponectin levels, type 2 diabetes risk, and obesity traits [44].

Our study also provides preliminary evidence for a novel peak on chromosome 8 for thigh circumference. Both targeted SNP and bioinformatic analysis of the 8q21.11-8q21.13 genomic region suggested *FABP4* (fatty acid binding protein 4, geneID 2167), which encodes a fatty acid binding protein that is highly expressed in adipose tissue and macrophages and is detected in high concentrations in serum. In children, *FABP4* variants were significantly associated with measures of insulin sensitivity, lipid metabolism and inflammation [48]. In conjunction with that evidence, our findings warrant further exploration of the role of *FABP4* in obesity pathogenesis.

Follow-up association analysis of variants under the linkage peaks identified five SNPs associated with adiponectin, percent body fat, thigh circumference, thigh skinfold, and waist circumference (Table 4). The genes tagged by three of these SNPs (rs730414 in *BTBD10* (BTB (POZ) domain containing 10, geneID 84280), rs1007750 in *SBSPON* (somatomedin B and thrombospondin, geneID 157869), and rs1596854 in *LUZP2* (leucine zipper protein 2, geneID 338645) have no obvious connections with obesity related phenotypes, although all are relatively newly identified and have not been studied extensively. The two remaining SNPs (rs2048417 and rs878953) are located in non-coding regions, and careful data mining discovered no indication of other types of known functional genomic elements.

Using bioinformatic analysis, we identified several putative candidate genes for the linkage peaks reported in this study. In addition to the previously discussed *FABP4*, we found *PPARGC1A* from the BMI, waist circumference, and body fat percent analyses, *SREBF2* from the adiponectin peak, and *PDX1* from the ghrelin peak especially noteworthy. *PPARGC1A* (peroxisome proliferator-activated receptor gamma coactivator 1 alpha, geneID 10891) encodes a master metabolic regulator previously implicated in insulin resistance [49]. Interestingly, a nonsynonymous polymorphism in *PPARGC1A* was linked to BMI in Polynesians, positioning *PPARGC1A* as a strong "thrifty gene" candidate in that population [50]. Other studies have implicated *PPARGC1A* expression and methylation in diet-induced metabolic changes, highlighting the biological relevance of our findings [49]. In the adiponectin analysis, the bioinformatic algorithm identified *SREBF2* (sterol regulatory element binding transcription factor 2, geneID 6721), an important transcription factor involved in cholesterol homeostasis that is downregulated in adipocytes from obese humans and upregulated following weight loss [51]. Finally, *PDX1* (pancreatic and duodenal homeobox 1, geneID 3651) emerged as a likely candidate from the ghrelin peak. *PDX1* encodes a transcriptional regulator that plays a critical role in insulin metabolism and is implicated in the monogenic form of early onset diabetes [52]. While its direct effects on the ghrelin concentrations remain to be tested in humans, a recent exome sequencing study discovered an association between a rare frameshift mutation in *PDX1* and the risk of type 2 diabetes in the general population [53]. It is worth noting that despite the implications for diabetes reported in other population, we did not find evidence of linkage in our previously published analysis of fasting glucose [22], likely due to the uniqueness of the Yup'ik population or chance.

We established that the association between genotype and two of the obesity traits (rs2048417 with adiponectin and

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rs730414 with body fat percent) is mediated by n-3 PUFA intake (Table 4). Previously, high levels of marine-derived n-3 PUFAs in the traditional Yup'ik diet have been suggested to play a role in the "healthy obesity" phenotype, in part, through gene-diet interactions [17,9]. The interaction of n-3 PUFA intake with the rs730414 genotype is particularly interesting. Although rs730414 is located in the intron of *BTBD10*, it is also in linkage disequilibrium with the gene encoding parathyroid hormone (*PTH*, geneID 5741). *PTH* plays a critical role in calcium homeostasis and is involved in vitamin D metabolism. The traditional diet of the Yup'ik people, which contains a large amount of marine-derived fatty acids, is also rich in vitamin D [54]. Additionally, variation in *PTH* and the gene encoding the vitamin D receptor (*VDR*, geneID 7421) has been tied to obesity-related traits. Therefore, the gene-diet interactions observed in our study are biologically plausible and warrant further investigation.

Our study has several important strengths. In addition to large sample size, an accurate biomarker of n-3 PUFA, and a wide range of n-3 PUFA intake, we were able to leverage data from extended family structures. Extended pedigrees provide one of the most powerful approaches for studying the genetics of quantitative traits, and the use of complex pedigrees that span multiple households and communities provides significant statistical power for discriminating between shared genetic and environmental effects. However, our findings must not be interpreted as causal for several reasons. First, the SNPs identified by targeted analysis may not represent the true susceptibility variant but rather "tag" the causal mutation. Second, the phenotypes that we examined are complex traits that are likely to be determined by a combination of genetic and environmental effects. Finally, the novel genomic regions for adiponectin and thigh circumference have not yet been replicated in other populations, and thus need further investigation. Additionally, given the genetic and environmental uniqueness of our study population, the generalizability of our findings may be limited, although replication analyses of our top hits in other Native groups are certainly warranted. Once successfully validated, these preliminary results will advance current understanding of the genetic and dietary determinants of obesity-related traits in communities with low prevalence of obesity-related comorbidities.

Author Contributions

LKV conducted bioinformatic analyses, interpreted the findings, and wrote the manuscript; HWW conducted linkage and association analyses; SA and DJL interpreted the findings and assisted in writing the manuscript; DBA, BB and HKT designed research, interpreted the findings, and edited the manuscript; PJH, KLS, DMO, and SEH conducted research. All authors approved the final version of the manuscript.

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Conflict of Interest

None of the authors has any conflict of interest.

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